J. Plant Develop.
20(2013): 51 – 55

IN VITRO RHIZOGENESIS IN PAPAYA (*CARICA PAPAYA* L.)

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Abstract: The seeds of two papaya (*Carica papaya* L.) cultivars ('Rainbow' and 'Sunrise Solo') were germinated on Murashige and Skoog (MS) medium with 3% sucrose, and free of plant growth regulators. Papaya contains some important secondary metabolites such as papain, and there would be interest in the *in vitro* mass production of papaya tissue of uniform origin. The most obvious form would be through the induction of somatic embryos, but rhizogenesis, an unexplored method, could provide as-yet unknown advantages. In this study, with the objective of artificaially inducing rhizogenesis *in vitro*, young leaves of both cultivars were placed on MS basal medium exposed to 5 concentrations (0, 1, 2, 4 or 8 mg/l) of auxins (2,4,5-trichlorophenoxyacetic acid, 2,4,5-T; indole-3-acetic acid, IAA; indole-3-butyric acid, IBA; α-naphthaleneacetic acid, NAA; β-naphthoxyacetic acid, BNOA) or phloroglucinol. All auxins could induce adventitious roots. Most roots (23/explant) formed with 2 mg/l NAA. The ability to induce only roots without any other intermediary organs such as callus or shoots provides an exclusive system for possible root-specific secondary metabolite production without the need for transgenic technologies such as *Agrobacterium rhizogenes*, or could provide a model protocol for more in-depth developmental studies on root development in papaya, an unexplored topic for this tropical plant.

Key words: leaf, Murashige and Skoog, paw-paw, roots, seeds.

Introduction

Papaya (*Carica papaya* L.; Caricaceae) is most frequently propagated by seed (reviewed in TEIXEIRA DA SILVA & al. 2007; JIMÉNEZ & al. 2014). Papaya seeds and other organs contain papain (fruit) and other secondary metabolites such as flavonoids and coumarin compounds in leaves [CANINI & al. 2007]. An *in vitro* protocol that would allow for the mass production of papaya tissue could benefit the commercialization of plants for products other than just the fruit. Several protocols for somatic embryogenesis in papaya exist (reviewed in TEIXEIRA DA SILVA & al. 2007; ANANDAN & al. 2012), as does a protocol for the photoautotrophic micropropagation of this tropical crop [TEIXEIRA DA SILVA, unpublished data]. The objective of this study was to induce rhizogenesis from young leaf tissue of seed-derived seedlings, which are vigorous and highly receptive *in vitro*. To date, no study has yet examined rhizogenesis in papaya.

Materials and methods

All auxins (PGRs) were purchased from Sigma-Aldrich (St. Louis, USA) and were of tissue culture grade. All other chemicals and reagents were of the highest analytical grade available and were purchased from Wako or Nacalai Tesque (Osaka, Japan), unless specified otherwise. Seeds of two hybrid papaya (*Carica papaya* L. cv. 'Rainbow' and

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'Sunrise Solo') cultivars were purchased from a local supermarket with guaranteed import quality and with no (or few) apparent surface infection or markings and were surface sterilized and germinated using the protocol of GIANG & al. (2011). Briefly, seeds were removed from ripe fruits, soaked for 48 h, washed in running tap water to remove the sarcotesta. Only sinking seeds following a floatation test (i.e., viable seeds) were used. Seeds were surface sterilized in 0.1% mercuric chloride ($HgCl_2$) + 2-3 drops of Tween-20 for 5 min, rinsed 3 times in sterilized distilled water (SDW), sprayed with 80% ethanol for 1 min then rinsed 3 times in SDW. Surface-sterilized seeds were slightly embedded (5/Petri dish) in autoclaved (100 KPa; 21 min) full-strength (macro- and micronutrients) MURASHIGE & SKOOG (1962) (MS) medium (pH 5.8) containing 3% sucrose and 2 g/l gellan gum (Gelrite®, Merck, USA). Petri dishes were sealed with Parafilm® and incubated at 25 °C under a 16-h photoperiod with a light intensity of 45 µmol/m^2/s provided by plant growth fluorescent lamps (Plant Lux, Toshiba Co., Japan). Young leaves of 10-day-old seedlings were cut at the junction with the stem, pooled, and exposed to 5 concentrations (0, 1, 2, 4 or 8 mg/l) of auxins (2,4,5-trichlorophenoxyacetic acid, 2,4,5-T; indole-3-acetic acid, IAA; indole-3-butyric acid, IBA; α-naphthaleneacetic acid, NAA; β-naphthoxyacetic acid, BNOA). The effect of phloroglucinol, which has strong auxin-like activity [TEIXEIRA DA SILVA & al. 2013], was also tested. After 30 days, the number of adventitious roots that formed from seed-derived seedling epicotyls was assessed. Experiments were organized in a randomized complete block design (RCBD) at 10 explants/treatment. All treatments and experiments repeated in triplicate. Data was subjected to analysis of variance (ANOVA) with mean separation by Tukey's multiple range test ($P \leq 0.05$; IRRISTAT version 3.0).

Results and discussions

Using the GIANG & al. (2011) protocol, the seeds of both papaya cultivars could be successfully sterilized. 100% germination was possible (Fig. 1A). Even though protocols for somatic embryogenesis in papaya exist (reviewed in TEIXEIRA DA SILVA & al. 2007a; ANANDAN & al. 2012), no study has yet examined rhizogenesis.

In this study, all auxins could induce roots (Fig. 1B-F). However, exposure to 2 mg/l NAA resulted in highest root formation (23.6/explant and 21.8/explant for 'Rainbow' and 'Sunrise Solo', respectively) (Tab. 1). For both cultivars, in the absence of any auxin, no roots formed. Except for IBA, in which rhizogenesis peaked at 2 mg/l for both cultivars, the other auxins showed a decreasing trend in terms of number of roots/explant and explant weight as the concentration of auxin increased from 1 to 8 mg/l (Tab. 1). IAA, IBA and 2,4,5-T induced more root hairs than the other auxins (Fig. 1D, 1E, 1F, respectively). PG, a known auxin-like compound [TEIXEIRA DA SILVA & al. 2013], produced few roots on explants (Tab. 1), but could increase the amount of adventitious roots on plantlets when cultured on Hyponex® medium (data not shown). The ability to induce only roots without any intermittent organs such as callus or shoots could be a simple yet effective way to mass produce root-specific secondary metabolites without using transgenic agents such as *Agrobacterium rhizogenes*, even though the transgenic route remains an important tool for other applications, such as the introduction of virus resistance to papaya [TENNANT, 2010]. This rhizogenic model could also allow for in-depth developmental analyses of root development in papaya. Auxin-induced rhizogenesis through the application of single doses of auxins, usually singly, was also possible in chrysanthemum thin cell layers [TEIXEIRA DA SILVA, 2003]. Thin cell layers provide an effective system for regeneration in many model plant systems [TEIXEIRA DA SILVA & DOBRÁNSZKI, 2013].

Tab. 1. Rhizogenic response of two papaya (*Carica papaya* L.) cultivars to auxins and PG.

Treatment	Conc. (mg/l)	Cultivar	No. roots/explant	Explant weight (mg)*
Control (no auxins)		Rainbow	0 h	44 f
		Sunrise Solo	0 h	47 f
NAA	1	Rainbow	14.2 b	224 b
	2		23.6 a	316 a
	4		8.7 cd	109 d
	8		0 h	39 f
	1	Sunrise Solo	11.2 bc	203 b
	2		21.8 a	286 ab
	4		7.9 cd	104 d
	8		0 h	42 f
IAA**	1	Rainbow	4.8 e	106 d
	2		2.6 fg	85 de
	4		1.1 gh	73 e
	8		0 h	38 f
	1	Sunrise Solo	4.1 ef	97 d
	2		1.8 g	78 e
	4		0.3 h	51 f
	8		0 h	40 f
BNOA	1	Rainbow	2.7 fg	91 d
	2		3.1 f	103 d
	4		1.3 gh	77 e
	8		0 h	40 f
	1	Sunrise Solo	2.1 g	88 de
	2		2.8 f	99 d
	4		0.4 h	56 f
	8		0 h	43 f
IBA**	1	Rainbow	6.7 d	93 d
	2		8.4 cd	114 cd
	4		2.6 fg	74 e
	8		0 h	44 f
	1	Sunrise Solo	7.1 d	104 d
	2		9.4 c	136 c
	4		4.2 ef	86 de
	8		0 h	44 f
PG	1	Rainbow	1.4 gh	76 e
	2		3.2 f	108 d
	4		2.1 g	74 e
	8		0 h	46 f
	1	Sunrise Solo	1.9 g	78 e
	2		3.6 f	132 c
	4		2.4 fg	91 d
	8		0 h	46 f
2,4,5-T**	1	Rainbow	8.2 cd	144 c
	2		4.1 ef	116 cd
	4		0.8 gh	72 e
	8		0 h	41 f
	1	Sunrise Solo	6.8 d	141 c
	2		2.3 g	108 d
	4		0.4 h	76 e
	8		0 h	40 f

Data presented as means (n = 30/treatment); different letters within a column across treatments and cultivars indicate significant differences ($P \leq 0.05$; Tukey's multiple range test). Auxin abbreviations: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; BNOA, β-naphthoxyacetic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, α-naphthaleneacetic acid. PG, phloroglucinol. * The basal weight of a cotyledonary explant = 36 mg (n = 30). ** Many root hairs formed on the roots induced by these auxins (see Fig. 1D, 1E, 1F)

Fig. 1. The qualitative response of papaya (*Carica papaya* L. cv. 'Sunrise Solo') to different auxins. (A) Sterilized seed germination and 10-day-old young leaves used for the study. Rhizogenesis in response to 1 mg/l of (B) NAA, (C) BNOA, (D) IAA, (E) IBA or (F) 2,4,5-T.

Conclusions

Rhizogenesis, defined in this study as the artificial induction of roots *in vitro*, was possible from young papaya leaves in response to several auxins, but not to phloroglucinol.

Acknowledgement

The author thanks Prof. Michio Tanaka for research support. The author declares no conflicts of interest (financial or other).

Abbreviations: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; BNOA, β-naphthoxyacetic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, α-naphthaleneacetic acid; PG, phloroglucinol; PGR, plant growth regulator.

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Received: 2 September 2013 / *Accepted*: 3 December 2013